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Persistent organochlorine pollutants in human serum of 50–65 years old women in the Flanders Environmental and Health Study (FLEHS). Part 2: correlations among PCBs, PCDD/PCDFs and the use of predictive markers

Adrian Covaci [a,*], Gudrun Koppen [b], Rudy Van Cleuvenbergen [b], Paul Schepens [a], Gerhard Winneke [c], Nicolas van Larebeke [d], Vera Nelen [e], Robert Vlietinck [f], Greet Schoeters [b]

[a] *Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610 Antwerpen (Wilrijk), Belgium*
[b] *Flemish Institute of Technological Research (VITO), Center of Expertise in Environmental Toxicology and Environmental Measurements, 2400 Mol, Belgium*
[c] *Medical Institute of Environmental Hygiene, Heinrich–Heine University, 40225 Düsseldorf, Germany*
[d] *Department of Radiotherapy, Nuclear Medicine and Experimental Cancerology, University of Ghent, 9000 Ghent, Belgium*
[e] *Department of Epidemiology, Provincial Institute of Hygiene, 2000 Antwerp, Belgium*
[f] *Centre of Human Genetics, University of Leuven, 3000 Leuven, Belgium*

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Abstract

In 1999, the FLEHS was set by the Flemish Ministry of Health, Belgium to assess pollutant concentrations and related health effect biomarkers in humans living in Flanders. Concentrations of selected organochlorine pesticides, polychlorinated biphenyls (PCB) and polychlorinated dibenzo-*p*-dioxins (PCDD) and furans (PCDF) were measured by gas chromatography–mass spectrometry and Chemical-Activated LUciferase gene eXpression (CALUX) bioassay in 47 serum pools of 200 women between 50 and 65 years living in two areas of Flanders. Correlation between TEQ values of different groups of compounds were computed in these pool results and it was found that total toxic equivalencies (TEQs) correlated well with the values of the groups of contributing compounds: mono-ortho PCBs ($r = 0.77$), non-ortho PCBs ($r = 0.65$) and PCDD/Fs ($r = 0.88$). The total TEQ was lower correlated to the CALUX–TEQ ($r = 0.57$). When calculating associations between those classes of compounds in the two studied regions separately, they were all higher correlated in the urban area compared to the more rural region. High correlation coefficients ($r > 0.80$) were also calculated between individual compounds and groups of compounds. It was suggested that in this studied background-exposed population, some compounds could be good predictors for a group: e.g. PCB 153 for indicator and total PCBs, PCB 118 for total PCB TEQ, PCB 156 for mono-ortho PCB–TEQs and total TEQ, 2,3,4,7,8-P_5CDF for PCDD/F TEQs and total TEQs. This means that in pooled serum samples correlations between persistent organochlorine compounds are as strong as for individual POP measurements observed in earlier studies.

Keywords: Polychlorinated biphenyls; Polychlorinated dibenzodioxins and furans; CALUX; Correlations; Markers; Human serum; Belgium

* Corresponding author. Fax: +32-3-820-2722.
E-mail address: covaci@uia.ua.ac.be (A. Covaci).

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1. Introduction

In 1999, the Flemish Environment and Health Study (FLEHS) was set up by the Flemish Ministry of Health, Belgium to assess exposure and related health effect biomarkers in humans living in Flanders (Staessen et al., 2001; Van Loon et al., in press). As part of the study, serum concentrations of organochlorine pesticides, polychlorinated biphenyls (PCB) and polychlorinated dibenzo-*p*-dioxins (PCDD) and furans (PCDF) were determined in a group of 50–65 years old women. Their concentrations and regional differences were presented in Koppen et al. (2002). This article describes the calculation of correlation coefficients between different single or groups of polychlorinated hydrocarbons measured in background-exposed women of two different regions. Both overall and regional diversified correlations were calculated. Correlations of $r = 0.59$–0.94 among persistent organochlorine compounds (POPs) were earlier shown among individual serum measurements (Koopman-Esseboom et al., 1994; Gladen et al., 1999; Longnecker et al., 2000). Here it was attempted to evaluate correlations and potential markers for classes of polychorinated aromatic hydrocarbons not in individual but in pooled serum samples.

2. Materials and methods

Complete details are given in the first paper of this study (Koppen et al., 2002). Summary information is presented below.

2.1. Study area and population

The rural area of Peer is situated 15–25 km from the nearest non-ferrous and chemical plants and lies away from motorways. The urban area (two suburbs of Antwerp city) is located 11–13 km SE from the chemical and petrochemical industry established in the harbour of Antwerp. The study group consisted of 200 healthy women between 50 and 65 years old from Antwerp ($n = 100$) and Peer ($n = 100$) recruited randomly between June and September 1999.

2.2. Sample collection and pooling procedure

Approximately 40 ml of blood was collected from each individual. Immediately after sampling, serum was separated and divided into one part for individual analysis of indicator PCBs congeners (3 ml) and CALUX–TEQ (2.5 ml) and another part for pooling. Pooling was done by ranking the women in the order of decreasing daily intake of meat and fish, decreasing daily intake of eggs and milk, increasing total number of weeks of breast feeding and increasing body mass index. The available serum of 3–5 subsequently listed individuals was pooled to ≈50 ml. Each of the 47 pooled samples was divided in three aliquots for the analysis of PCDD/PCDFs (25 ml), PCBs/organochlorine pesticides (13 ml) and CALUX–TEQ (4 ml).

2.3. Analyses of polychlorinated aromatic hydrocarbons

In 47 pooled serum samples, mono-ortho PCBs (PCB 105, 118, 156, 157, 167), indicator PCBs (28, 52, 101, 138, 153, 180) and PCB 44, 66, 74, 99, 110, 128, 149, 170, 183, 187, 194, 199 were measured. The pooled samples were also analysed for the non-ortho PCBs (77, 81, 126, 169) and the 17 PCDD/PCDF toxic congeners. The analysis of mono-ortho, non-ortho PCBs and PCDD/PCDF congeners allowed the calculation of the toxicity equivalents (TEQ) for each sample using the TEF scheme of the WHO (Van den Berg et al., 1998). The chemical analysis methods have been previously described in detail (Koppen et al., 2002). The CALUX bioassay was a variant based on a previously described procedure (Murk et al., 1998).

2.4. Statistical analysis

Database management and statistical analysis were performed with Statistica version'99 (Statsoft Inc.). Non-normally distributed data were log-transformed. Regression analysis of marker substances or alternative measurements on the one hand vs. measured groups of organochlorines was performed. Using the best equation obtained in the regression models, the difference between estimated and observed concentrations were calculated.

3. Results and discussion

3.1. Relationships between TEQs of organochlorines in pooled serum samples

Longnecker et al. (2000) reported that for populations with background-exposure (like in our case), it is impossible to sort out the possible contribution of the various organochlorine compounds to health effects because of their strong associations. In Table 1, Pearson correlation coefficients between the different TEQ values in the 47 pooled serum samples are given. The total WHO–TEQ value was in good correlation with the values of the individual contributors: mono-ortho PCBs ($r = 0.77$), non-ortho PCBs ($r = 0.65$) and PCDD/Fs ($r = 0.88$) considering all serum pools in both regions (Table 1a). TEQ values from non-ortho PCBs were poorly correlated ($r = 0.23$) with PCDD/F–TEQs. This could indicate that, beside main intake through the diet, these two groups of POPs might have different exposure sources. A better correlation was found with

Table 1
Pearson correlation coefficients between the different TEQ measurements and between TEQs and indicator PCBs, for both regions together (a) and separated per region; (b, c) (all log-transformed, except for the CALUX values)

TEQ	MO-PCB	NO-PCB	MO + NO-PCB	PCDD	PCDF	PCDD/F	Total TEQ	IND-PCB
(a) P + A: $N_{pool} = 47$								
Calux	0.39**	0.53***	0.51***	0.38**	0.34**	0.43**	0.57***	0.24
MO-PCB	–	0.65***	0.86***	0.52***	0.35*	0.49***	0.77***	0.84***
NO-PCB		–	0.95***	0.21	0.20	0.23	0.65***	0.41**
MO + NO PCB			–	0.34*	0.27	0.34*	0.75***	0.62***
PCDD				–	0.35*	0.76***	0.69***	0.52***
PCDF					–	0.87***	0.76***	0.43**
PCDD/F						–	0.88***	0.55***
Total TEQ							–	0.70***
(b) P: $N_{pool} = 22$								
Calux	0.24	0.36	0.34	0.52*	0.17	0.39	0.43*	0.07
MO-PCB	–	0.67***	0.86***	0.51*	0.06	0.29	0.57**	0.78***
NO-PCB		–	0.95***	0.37	0.16	0.30	0.62	0.42*
MO + NO PCB			–	0.45*	0.13	0.32	0.64**	0.61**
PCDD				–	0.87***	0.62**	0.66**	0.32
PCDF					–	0.87***	0.75***	0.25
PCDD/F						–	0.92***	0.34
Total TEQ							–	0.52*
(c) A: $N_{pool} = 25$								
Calux	0.61***	0.76***	0.79***	0.32	0.50*	0.46*	0.73***	0.39
MO-PCB	–	0.53**	0.79***	0.52**	0.70***	0.68***	0.88***	0.84***
NO-PCB		–	0.93***	0.02	0.29	0.17	0.60**	0.26
MO + NO PCB			–	0.22	0.48*	0.39	0.79***	0.53**
PCDD				–	0.55**	0.87***	0.70***	0.62**
PCDF					–	0.89***	0.84***	0.66***
PCDD/F						–	0.87***	0.71***
Total TEQ							–	0.77***

P: Peer, A: Antwerp, IND-PCB: indicator PCB, NO-PCB: non-ortho PCB, MO-PCB: mono-ortho PCB, Total TEQ: sum TEQ values of mono-ortho PCB, non-ortho PCB, PCDDs and PCDFs.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

mono-ortho PCBs ($r = 0.65$). TEQ values from mono-ortho PCBs also showed a higher correlation with PCDD/F ($r = 0.49$). All correlation coefficients calculated from these data were similar with those found for individual measurements in Dutch mothers by Koopman-Esseboom et al. (1994) and Gladen et al. (1999) or Longnecker et al. (2000) in individuals of a Michigan and Canadian population respectively. This suggests that correlations among classes of TEQs are not lost in pooled serum data.

3.2. Single markers of exposure

The distorted measure of effect—due to high correlations between organochlorine compounds—may be considered advantageous, because less expensive measurements can be used as a proxy for estimation of e.g. total TEQ (Longnecker et al., 2000). It was shown that the analysis of even single marker substances in serum could provide cost-effective assessment of human exposure to complex mixtures of organochlorines (Glynn et al., 2000). Strong relationships were found between the concentrations of single mono-ortho and di-ortho PCBs and groups of PCB congeners in serum (Table 2). The preferential analysis of only these compounds (preferably PCBs) is possible by the use of fast analytical methodologies (Covaci and Schepens, 2001). When correlation coefficients were higher than $r = 0.75$, linear regression was performed between the concentration of the potential marker substance and groups of polychlorinated aromatic hydrocarbons. The difference between observed and estimated concentration for each serum sample of indicator PCBs, mono-ortho PCB TEQs, PCB TEQs, PCDD/F TEQs and total TEQs are given (Fig. 1). The estimated concentration was calculated using the concentration of single marker sub-

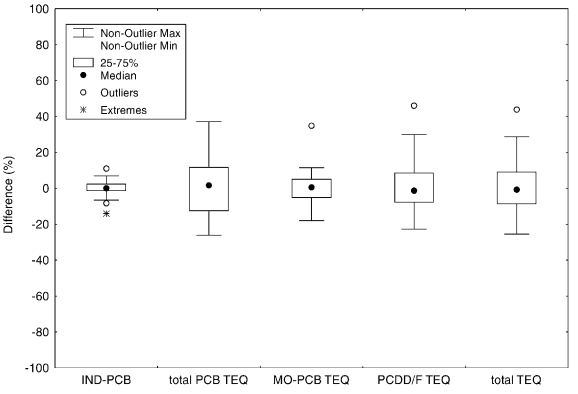

Fig. 1. Box plots of percentage difference between observed and estimated concentration in each sample of indicator PCBs (IND-PCB), total PCB TEQ, mono-ortho PCB TEQ (MO-PCB TEQ), PCDD/F TEQ and total TEQ. The estimated concentration was calculated using the concentration of single marker substances: PCB 153 (for indicator PCBs), PCB 118 (total PCB TEQ), PCB 156 (mono-ortho PCBs), 2,3,4,7,8-P_5CDF (PCDD/F TEQ and total TEQ). Outliers = if data point value > UBV + 1.5(UBV − LBV), or data point value < LBV − 1.5(UBV − LBV), with UBV and LBV the upper and lower value of the box.

Table 2
Pearson correlation coefficients between single markers and polychlorinated aromatic hydrocarbon compound groups (all log-transformed). For each group and single markers, the best correlation coefficients was highlighted

Single marker	Compound group							
	IND-PCB	PCB total	PCB total TEQ	MO-PCB TEQ	PCDF-TEQ	PCDD-TEQ	PCDD/F TEQ	Total TEQ
PCBs								
PCB 153	**0.99*****	**0.96*****	0.60***	0.83***	0.43***	0.53***	0.55***	0.69***
PCB 180	0.92***	0.89***	0.57***	0.76***	0.47***	0.50***	0.57***	0.68***
MO-PCBs								
PCB 118	0.63***	0.74***	**0.83*****	0.82***	0.21	0.33*	0.30*	0.64***
PCB 156	0.80***	0.82***	0.77***	**0.94*****	0.35*	0.55***	0.51***	0.73***
PCDD/F								
2,3,4,7,8-P_5CDF	0.52***	0.47**	0.25	0.40**	**0.97*****	0.39**	**0.87*****	**0.75*****
1,2,3,7,8-P_5CDD	0.43**	0.37*	0.18	−0.14	0.35*	**0.91*****	0.72***	0.58***

IND-PCB: indicator PCB, MO-PCB: mono-ortho PCB, Total TEQ: sum TEQ mono-ortho PCB, non-ortho PCB, PCDDs and PCDFs.

$^{*}p < 0.05$.

$^{**}p < 0.01$.

$^{***}p < 0.001$.

stances: PCB 153 (for indicator PCBs), PCB 118 (total PCB TEQ), PCB 156 (mono-ortho PCBs), and 2,3,4,7,8-P_5CDF (PCDD/F–TEQ and total TEQ). All of them were good markers with more than 50% of the pooled serum samples having a difference lower than 15% between observed and estimated concentrations of the respective compound groups' concentrations.

3.3. Regional differences in correlations between organochlorines

Comparative to the rural region, higher correlation coefficients were found in the urban area between almost all TEQ values and between TEQ values and indicator PCBs (Table 1 (panels b and c)).

Total WHO–TEQ, PCDD/PCDF–TEQ and CALUX–TEQ values in the pooled serum samples were not different for the women living in both regions. The main intake route for POPs is considered to be the food consumption. There was no difference in reported food consumption in both regions, except for local food, which was more consumed in the rural area (70% of women reported local food consumption vs. 28% in Antwerp) (Koppen et al., 2002). However, higher PCB concentrations in the urban area Antwerp were observed (see Koppen et al., 2002 for more detailed results). This suggests intake from PCBs from higher industrial activities in Antwerp favoured the better correlation between the different classes of organochlorine compounds.

The measurement of indicator PCBs and CALUX–TEQ can be done without the need for large volumes of serum. Their overall correlation coefficients with the total TEQs were $r = 0.70$ and 0.57 respectively (Table 1 (panel a)). Both increased to 0.77 and 0.73 when considering only the women in the urban region (Table 2 (panel c)). The latter correlation layed in the range of what was found by comparing GC/MS determined total WHO–TEQs (including PCBs) and CALUX–TEQ in: human serum, $r = 0.71$ (Aarts et al., 1996) and cow's milk, $r = 0.74$ (Bovee et al., 1998). For the relations: CALUX–TEQ $\Longleftrightarrow$ total TEQ and indicator PCB $\Longleftrightarrow$ total TEQ, regression equations were calculated. The respective equations were very similar if calculated for all samples of the women in Peer and Antwerp as well as for both regions seperatly. Using those three different regression lines, the concentrations of total TEQ for all 47 serum samples was estimated from their concentrations of CALUX–TEQ or indicator PCBs (Fig. 2a and b). This was done to find out if the regional differences in correlation were of large influence on the predictive value for total TEQ of these two examined marker measurements. Estimating the mean total TEQ based on

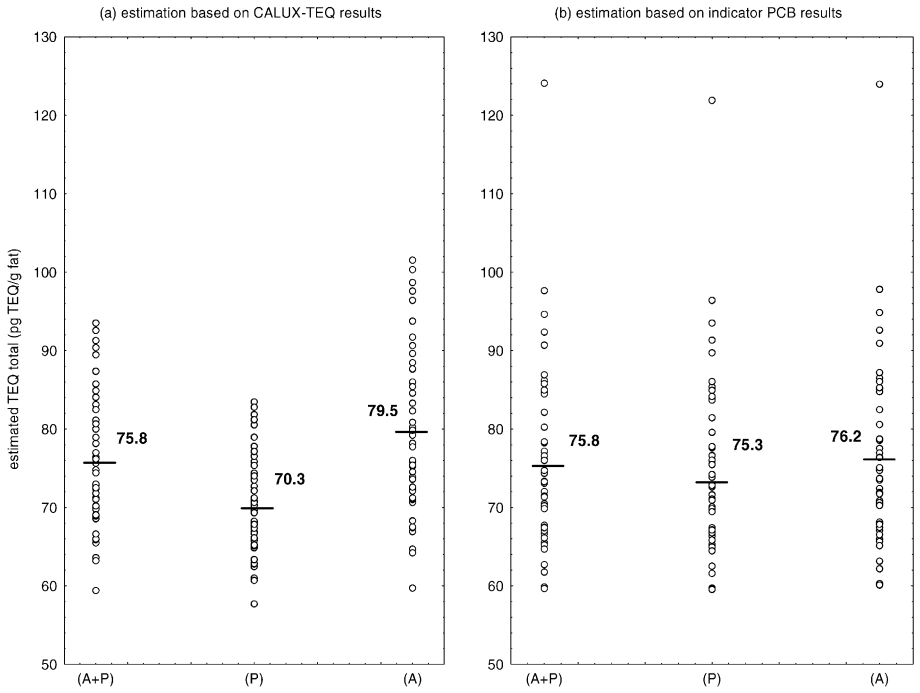

Fig. 2. Estimation of total TEQ for all 47 pooled serum samples from measured (a) CALUX–TEQ results and (b) indicator PCB results. This was done using the overall regression equations obtained for individual samples in both regions (A + P), or those for the region Peer (P) or Antwerp (A). The experimental TEQ values in the pooled samples were: 72.7, 72.6 and 75.1 pg TEQ/g fat for A + P, P and A, respectively.

marker PCB results there was no difference whether using the regression found in Peer (75.3 pg TEQ/g fat), Antwerp (76.2 pg TEQ/g fat) or overall (75.8 pg TEQ/g fat). Moreover, the estimated values were statistically identical with the measured value of 75.0 pg TEQ/g fat (Koppen et al., 2002). However, mean total TEQ values calculated based on CALUX–TEQs using the three regression lines were 70.3, 75.8 and 79.5 pg TEQ/g fat respectively. The first value was significantly different from the others ($p < 0.001$), which could be expected based on the rather low correlation coefficient found between CALUX–TEQs and total TEQ in the rural area ($r = 0.43$). However all three equations lead to a reasonable equal predicted total TEQ value within certain confidence range. If another population would be examined with background contamination of POPs, it would be therefore possible to 'predict' TEQ values—within a confidence interval—by measuring indicator compounds. This opens the possibility to estimate the total TEQ values from measurements that are easier and cheaper and need lower amounts of blood.

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